J Appl Electrochem (2013) 43:237–244
DOI 10.1007/s10800-012-0507-0

ORIGINAL PAPER

Biocides electrogeneration for a zero-reagent on board disinfection of ballast water

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Received: 31 July 2012 / Accepted: 5 November 2012 / Published online: 20 November 2012

Abstract The feasibility of a quick electrochemical process for on board zero-reagent treatment of ballast water by anodic and cathodic production of oxidants was proposed. The process has been tested in the inactivation of the marine dinoflagellates *Alexandrium minutum* and *A. taylori*, both responsible for algal blooms and toxin-producing, and against the marine bacterium *Pseudomonas aeruginosa*, a Gram-negative pathogenic micro-organism. A complete inactivation of both dinoflagellates was quickly achieved with electro-generated active chlorine, while higher resistance to oxidising agents was verified for *P. aeruginosa*. A combined sequential treatment involving anodic oxidation followed by extended exposure time in the absence of current, and a final cathodic treatment was proposed. The cathodically electro-generated hydrogen peroxide contributed to the reduction of treatment time and the removal of residual species.

Keywords Ballast water · Dinoflagellates · *Pseudomonas aeruginosa* · Boron-doped diamond · Active chlorine · Hydrogen peroxide

Electronic supplementary material The online version of this article (doi:10.1007/s10800-012-0507-0) contains supplementary material, which is available to authorized users.

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1 Introduction

The invasion of coastal areas by non-indigenous species introduced by the common ballast water disposal practice has become, in the last decades, a serious environmental problem. It is estimated that the amount of ballast water carried in ships is more than 10 billion tons per year [1]. Several studies have identified a wide range of organisms, including pathogenic bacteria, such as *Vibrio cholerae* and *Escherichia coli* [2, 3], and different species of dinoflagellates, such as *Alexandrium catenella*, *Gymnodinium catenatum* and *Protoceratium reticulatum* [4, 5]. Though most organisms taken onboard during ballast intake do not survive the voyage or fail to establish viable populations once discharged [6], several bacteria and microalgae have established and dominated the receiving coastal areas, as widely documented in literature [4, 7–10]. To reduce the human health risks, according to the International Convention for the Control and Management of Ships' Ballast Water and Sediments adopted by the International Maritime Organisation (IMO) [11], the need exists, therefore, to introduce safe and efficient technologies for ballast water control and treatment. At present, however, few technologies have been shown to be biologically effective, environmentally friendly, safe and suitable for onboard use in all the temperature range and for all the species to be considered. The first ballast water management strategy involved the open ocean exchange, but this practice is only 90–95 % effective and can be dangerous in foul weather or can produce excessive hull stress [12].

Numerous treatment technologies have been proposed, including filtration [13], heating treatment [14], ultraviolet radiation, and several chemical disinfection treatment involving the use of biocides and active substances, such as chlorine, hydrogen peroxide, chlorine dioxide and ozone

[15, 16]. Though the disinfection effectiveness of chemical systems is generally high, they need to meet several requirements. In particular, the oxidizing agents should show a broad spectrum effectiveness, and, at the same time, they should be safe to handle, non-corrosive and not generating dangerous by-products, to avoid the potential release of harmful substances into the environment [4].

As a result of these disadvantages a number of alternatives are commonly investigated. The alternatives are wide ranging including physio-chemical systems, such as titanium photocatalysis, photodynamic disinfection, electrochemical disinfection, and physical systems, ultrasonication, pulsed electric fields, irradiation, magnetic enhanced disinfection, and microwave systems [16].

Among them, electrochemical disinfection of ballast water has emerged as the most promising alternative to chemical disinfection, due to the possibility of onsite generation of oxidants thus reducing the handling and storing hazards associated with chemicals [1]. Previous studies have assessed the feasibility of hydrogen peroxide generation in marine water in divided reactors to inactivate biological species [17], also indicating the occurrence of the reaction between active chlorine and hydrogen peroxide as the limiting factor of oxidants accumulation [18].

Considering the ballast waters uniqueness, experimental feasibility studies are needed to evaluate the optimal treatment conditions and to investigate both cell configuration and electrode materials. The inactivation efficacy of electrochemical disinfection systems is in fact largely dependent on cell configuration, electrode material, electrolyte composition, micro-organism characteristics and other experimental parameters, such as flow rate and current density [4].

The present research aims at simultaneous production of different oxidants through the development and optimisation of a combined treatment involving a sequential anodic and cathodic process. Boron-doped diamond (BDD) was used as the anode, to ensure hydroxyl radicals production without the excessive chlorine and active chlorine electrogeneration [19]. In addition, though BDD requires high investment cost, it ensures a very high reproducibility and durability, even in complex environmental matrixes [18]. A gas diffusion cathode (GDE) was used, since it has been verified that such an electrode ensures high current efficiency in a wide range of current density, even when air is used [20]. The process has been tested for the inactivation of marine toxin-producing dinoflagellates responsible for algal blooms, such as *A. minutum* Halim and *A. taylori* Balech. The selected species may form cysts that give them high resistance to transport conditions. The marine bacterium *Pseudomonas aeruginosa,* a Gram-negative ubiquitarius and versatile micro-organisms strain, known to be pathogenic for several organisms included humans, was also treated.

Direct epifluorescence microscopy and plating technique were used to evaluate the viability of organisms under different treatments. In particular, specific fluorochromes staining differently live and dead cells were used and observed under different light excitation and emission wavelength [21–24].

It is opinion of some authors [25–27], that the use of dyes does not always provide reliable answers on the vitality of algal cells in relation to the disinfection system used. Taking this in account in this study, for marine dinoflagellates the natural red autofluorescence (RAF) of chlorophyll was also used as viability indicator [28, 29], while white autofluorescence (WAF) due to bleaching was used as a dead cells indicator.

2 Experimental

2.1 Materials

Artificial seawater was prepared according to Kester et al. [30] dissolving 23.926 g kg^{-1} NaCl, 4.008 g kg^{-1} Na_2SO4, 0.677 g kg^{-1} KCl, 0.196 g kg^{-1} $NaHCO_3$, 0.098 g kg^{-1} KBr, 0.026 g kg^{-1} H_3BO_3, 0.003 g kg^{-1} NaF, 5.08 g kg^{-1} $MgCl_2$, 1.1477 g kg^{-1} $CaCl_2$, 0.0142 g kg^{-1} $SrCl_2$. The solution obtained presented an initial pH of 8.0–8.2 and a conductivity of about 45 mS.

All reagents were supplied by Carlo Erba Company and Sigma-Aldrich and used in their commercially available form with no further purification.

Non-axenic cultures of *A. minutum* Halim and *A. taylori* Balech were provided by OGS-Istituto Nazionale di Oceanografia e di Geofisica Sperimentale, Trieste, Italy. Cultures were grown in a f/2 medium [31], without silica addiction in a culture chamber at 19 ± 1 °C with a 12:12 light:dark cycle and a 100 µmol photons m^{-2} s^{-1} irradiation.

Pseudomonas aeruginosa ATCC 13388 was obtained from the American type culture collection (ATCC-Rockville-Maryland-USA). The bacterial strain was revitalized on specific medium-*Pseudomonas* agar base (PAB) (Oxoid LTD, Basingstoke, England) at 37 °C—and then cultured for the treatment tests on Marine Broth (Carlo Erba, Milano Italia) in the dark for 24 h on a Certomat®U-Braun, a benchtop shaker programed to operate at 120 m^{-1} at room temperature.

2.2 Electrochemical treatment procedure

Electrolyses were performed under galvanostatic conditions using an AMEL 2051 potentiostat. A thermostated 200 mL two-chamber cell stirred with a magnetic bar was used. The two compartments, were separated by a cation

exchange membrane (Nafion® 324, Sigma-Aldrich). A commercial BDD electrode (supplied by CSEM) was used as the anode. The working electrode was an uncatalysed air fed gas diffusion electrode (GDE), coated on both sides by hydrophobic Shawinigan acetylene black carbon provided by Industrie De Nora (Milan, Italy).

The electrodes had a geometric area of 5 cm^2 and were placed vertically at a distance of about 1 cm.

The current efficiency, defined as the ratio of the hydrogen peroxide produced to that theoretically obtained by means of Faraday's law, was calculated according to Eq. 1:

$$\mathrm{CE}(\%) = \frac{nF[\mathrm{H_2O_2}]_{\mathrm{exp}}}{Q \times MW \times 10^4} \times 100 \quad (1)$$

where n is the number of equivalents, F is the Faraday constant (96485 C mol^{-1}), Q the charge (coulomb) at a given time t, MW the hydrogen peroxide molecular weight.All the experiments were conducted in triplicate.

Dinoflagellate species were treated with active chlorine electro-generated in a range of current density and treatment time of 50–100 A m^{-2} and 15–30 min, respectively.

Pseudomonas aeruginosa cultures were treated in a range of current density of 100–150 A m^{-2} varying time of exposition, and adding permanence (including a residence time) in the electrolytic cell at the end of the electrolysis.

The experimental conditions are summarized in Table 1.

For each test an initial culture of 50 mL was transferred in the electrolytic cells and was quickly diluited 1:2 with sterile water. After the treatment subsamples were collected to assess the live cell% (three replicates) and to evaluate the capacity to regrowth on specific medium, forming colonies (up to four plates).

In order to remove residual traces of active chlorine, hydrogen peroxide, and other radical species that could interfere with microscopy investigations and regrowth trials, after each treatment the dinoflagellates culture samples were centrifuged at 250 g for 10 min and the pellet were resuspended in the specific culture medium to proceed with the staining and microscopic observation. *P. aeruginosa* samples were centrifuged two times and resuspended in physiological solution.

Subsamples of dinoflagellate treated cultures, were placed into the culture chamber to evaluate the viability of inoculum after a week. Instead, 30 µL *P. aeruginosa* treated cultures were plated on two–four recovery plates PAB and incubated at 37 °C in the dark, in order to verify the regrowing ability in a very favourable condition.

Table 1 Summary of experimental conditions

Treatment	Current density (A m^{-2})	Electrolysis time (min)	Contact time after electrolysis (min)	Cathodic treatment (min)
T1	50	15	–	–
T2	100	15	–	–
T3	100	30	–	–
T4	100	60	–	–
T5	150	15	–	–
T6	150	30	–	–
T3-30	100	30	30	–
T3-60	100	30	60	–
T3/C	100	30	–	30
T3-30/C	100	30	30	30

2.3 Analysis

pH was measured using a Crison GLP 421 pH metre; conductivity was measured using a HD9213-R1Delta Ohm metre. Hydrogen peroxide concentration was determined reflectometrically by means of Merck specific analytical test based on a peroxidase reagent.

The active chlorine concentration was determined using the DPD (*N*,*N*-diethyl-*p*-phenylenediamine) colorimetric method in accordance with EN ISO 7393/2 method (absorbance peak at 510 nm measured by means of a PG Instruments T80 + UV/Vis spectrophotometer and quartz cells of 1 cm path length).

Anions were determined using a Dionex 120 ionic chromatograph equipped with an IONPAC AS12A anionic column in isocratic mode and conductometric detector.

2.4 Staining and microscopic observation

CalcoFluor White M2R (CFW) was used to stain thecal plate of dinoflagellates and determine culture density (n cell mL^{-1}). In order to establish the viability of cultures under different treatments, the specific Fluorochrome Fluorescein DiAcetate (FDA) (F1303, Molecular Probes, Invitrogen), that stains only alive cells with metabolic activities [32–34], was used and compared with natural RAF of chlorophyll. WAF due to bleaching was used as dead cells indicator.

Initial *P. aeruginosa* culture abundance (bacterial cell mL^{-1}) was determined by direct counting using DAPI (4,6-diamidino-2-phenylindole) as DNA staining agent. Cell viability before and after the treatments was determined by two-dye fluorescent bacterial viability kit (Kit Live/Dead® Bacterial Viability Kit, BacLight™ Molecular Probes, Inc., Eugene, OR, USA) [31, 35]. This technique is based on different permeability of dyes to the micro-organism membrane. Live is a green nucleic acid dye that stains both intact and compromised membrane cells, when used alone, while Dead is a red nucleic acid dye cell-impermeable that labels only cells with compromised membrane causing also

a reduction in the green stain fluorescence when both dyes are present. Thus, viable bacteria stain green while dead bacteria stain red. The cells viability was expressed as the percent of alive cells compared to total number cells.

Three samples of each culture were prepared for microscopy analysis performed by an epifluorescent inverted microscope (AXIOSKOP 40, Zeiss, Germany) equipped with a mercury lamp (HBO 50/AC), 100X magnification and four excitation filters to observe and to count cells at different wavelength emission, specific for staining and auto-fluorescence detection (Table 2).

Images were captured with a digital camera (Zeiss Axiocam MCR, Axio Cam MRm, Zeiss, Germany) and processed with Axio Vision software (Axio Cam MRm, Zeiss, Germany).

3 Results and discussion

3.1 Biocides electrogeneration

To evaluate the feasibility of an electrochemical inactivation of the selected marine micro-organisms, several preliminary tests of biocides electro-generation were conducted in a divided reactor.

At the cathode, the use of an air fed GDE electrode resulted in the reduction of the molecular dissolved oxygen with hydrogen peroxide production according to the two-electron reaction:

$$O_{2(g)} + 2H_3O^+ + 2e^- \rightarrow H_2O_2 + 2H_2O \quad (2)$$

In particular, under the alkaline conditions provided by seawater, the reduction of molecular oxygen led to the formation of hydroperoxide ion that represents the conjugate base of hydrogen peroxide:

$$O_{2(g)} + H_2O + 2e^- \rightarrow HO_2^- + OH^- \quad (3)$$

At the same time, different parasite reactions are known to contribute to the decrease in the hydrogen peroxide concentrations, mainly the four-electron reduction to water and the hydrogen evolution, both enhanced by increasing values of pH and current density. Moreover, spontaneous decomposition to water and hydrolysis reaction to water and oxygen especially under alkaline conditions can occur.

As the seawater pH is high and well buffered, due to the presence of a large amount of salts, hydrogen peroxide production has been maximised by studying the effect of only the current density in the range 50–150 A m^{-2}.

The concentrations of hydrogen peroxide produced along time at different current densities are reported in Fig. 1a.

The results indicate that hydrogen peroxide concentrations increased linearly with electrolysis time with a generation rate in the order $50 < 100 < 150$ A m^{-2}. In particular, a slope of 1.3544, 2.6157 and 3.413 was observed for 50, 100 and 150 A m^{-2}, respectively, with a correlation coefficient always greater than 0.99. A progressive loss of efficiency at increasing reaction time is also indicated by inset of Fig. 1a, probably due to the simultaneous occurrence of different cathodic side reactions. As the current efficiency values found in the runs conducted at 50 and 100 A m^{-2} were quite similar, at least in the first 60 min of electrolysis, 100 A m^{-2} represented the best compromise between time and yield.

Table 2 Excitation and emission wavelength for fluorescence detection of fluorochromes and natural autofluorescence

Staining	Excitation (nm)	Emission (nm)
CFW, RAF, WAF, DAPI	530–585	615
FDA, live/dead	450–490	515
Dead	545/25	605/70
FDA, live	470/40	525/50

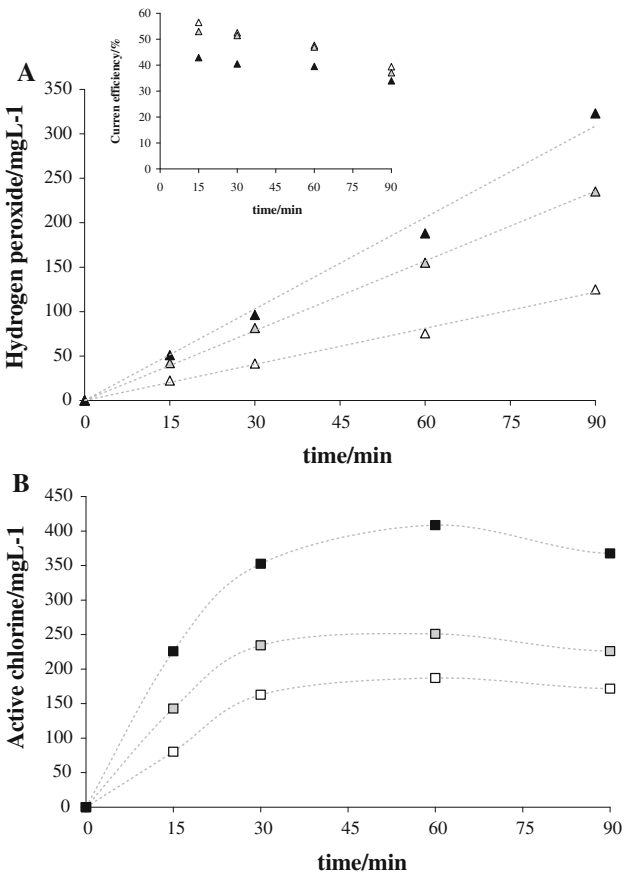

Fig. 1 Effect of current density with time on H_2O_2 (**a**) and active chlorine (**b**) in electrolyses of seawater performed at 50 (*white symbols*), 100 (*grey symbols*) and 150 (*black symbols*) A m^{-2}. $T = 23 \pm 1$ °C. *Inset* current efficiency of H_2O_2 production with time

The adoption of a BDD anode lead to the simultaneous production of active chlorine, a mixture of oxidants including Cl_2 hypochlorite ion ClO^- and hypoclorous acid with strong and well-known biocide power:

$$2Cl \rightarrow Cl_2 + 2e^- \quad (4)$$

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^- \quad (5)$$

$$HOCl \rightarrow OCl^- + H^+ \quad (6)$$

As can be seen in Fig. 1b, increasing time and current densities caused an increase in active chlorine concentrations. In particular, the curves, after a rapid initial growth, reached a limit concentration which remained almost unchanged even at times much higher than those adopted in the tests (data not shown). More in detail, electrolyses conducted at 50 and 100 A m^{-2} attained the maximum value of about 163 and 234 mg L^{-1} after 30 min, while 60 min electrolyses were required to reach the highest concentration of 409 mg L^{-1} in the experiments conducted at 150 A m^{-2}.

Active chlorine values were lower than expected especially considering that the sea water contains large amounts of chlorides (about 20 g L^{-1}). The data obtained can be attributed to a negative effect of pH, which quickly dropped during the treatment, as shown in Fig. 2, from an initial value of 8.2–6.3, 4.4 and 3.5 for 50, 100 and 150 A m^{-2}, respectively.

The equilibrium of the active chlorine species, in fact, is strongly affected by pH: under strong acidic conditions (pH < 3.3), Cl_2 (aq) is favoured, thus determining active chlorine depletion caused by Cl_2 volatilization.

3.2 Micro-organism inactivation

Preliminary chemical tests (data not shown) verified that active chlorine was more effective than hydrogen peroxide in the inactivation of all the selected micro-organisms including *P. aeruginosa*, that showed a meaningful resistance to the biocide action of hydrogen peroxide. Due to the constraints imposed by the time requirement of an on board treatment, the need exists to quickly achieve high treatment efficiencies. For this reason the disinfecting action of active chlorine was first investigated.

Figure 3 shows the cell densities of dinoflagellate cultures determined on samples differently stained. CFW tests highlighted the total number of cells by staining the empty thecae, and both the viable and dead cells. The number of viable cells was further determined by RAF and FDA. As it can be noticed, the data did not match, and cells densities determined by FDA were lower than that detected by RAF, thus suggesting that some FDA negative cells should be in a quiescent or inactive phase as cysts [36].

Finally, as expected, very low values were found for WAF densities, indicating low amounts of dead cells. In addition, a higher density of viable cells for the samples of *A. minutum* compared to *A. taylorii* was observed.

Dinoflagellate species were treated with current densities of 100 A m^{-2} for 30 and 15 min (treatments T3 and T2), and 50 A m^{-2} for 15 min (treatment T1), that corresponded to final concentrations of active chlorine of about 250, 150 and 85 mg L^{-1}, respectively. As shown in Fig. 4a, b, all the electrochemical treatments tested caused cells death and both the species showed similar response.

No cells were detected by either FDA or RAF staining, while they were characterized only by WAF. A reduced number of total cells was finally detected, due to cellular lysis well highlighted by CFW staining. 1 week after recovery the cultures appeared undeveloped with only dead cells characterized by WAF (data not shown).

The data obtained suggest that *A. minutum* and *A. taylori* showed high sensibility to the anodic treatment on BDD anodes. Complete cells inactivation was observed even at low-current density and short treatments. This unexpected high efficiency can be attributed to the synergic effect of current, active chlorine concentration and hydroxyl radicals

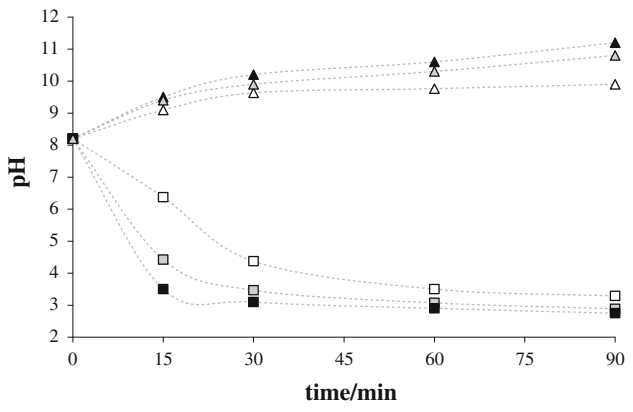

Fig. 2 pH evolution of anolyte (*squares*) and catholite (*triangles*) in electrolyses of seawater performed at 50 (*white symbols*), 100 (*grey symbols*) and 150 (*black symbols*) A m^{-2}. $T = 23 \pm 1$ °C

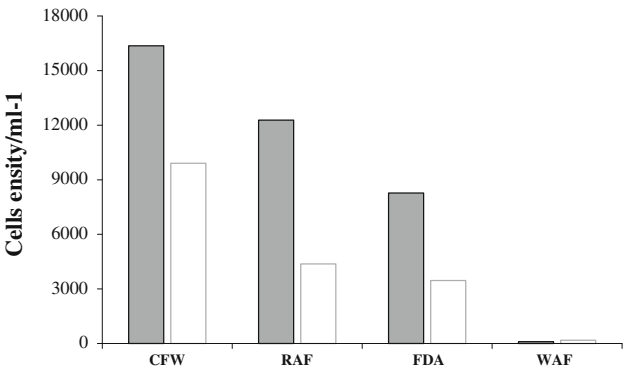

Fig. 3 Cells density of untreated *A. minutum* (*grey column*) and *A. taylorii* (*white column*)

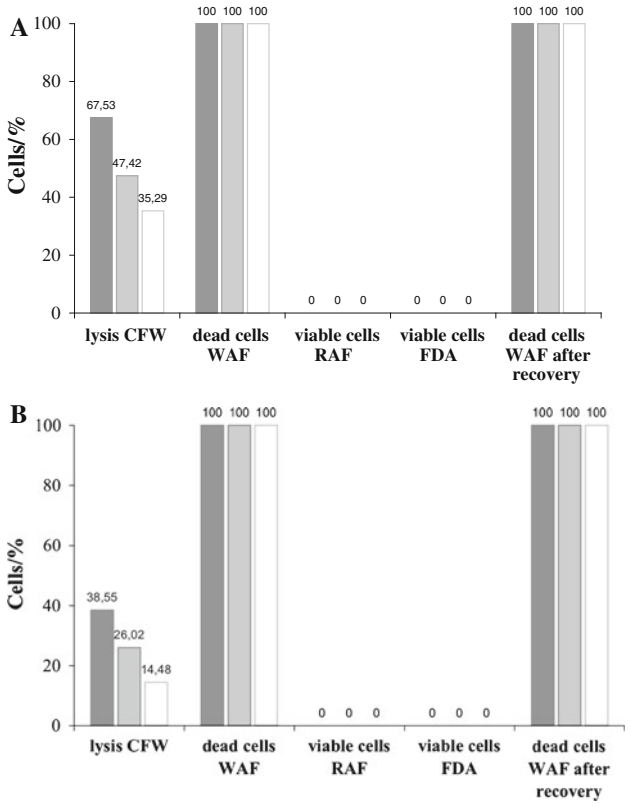

Fig. 4 Cells percentage of *A. minutum* (**a**) and *A. taylori* (**b**) detected with different staining after electrochemical treatment: *j* 100 A m^{-2}, *t* 30 min (*black column*); *j* 100 A m^{-2}, *t* = 15 min (*grey column*); *j* 50 A m^{-2}, *t* = 15 min (*white column*). *T* = 23 ± 1 °C

formed during the process. A crucial role may be also played by pH. Under the established acidic conditions the species survival was strongly affected and, in addition, the equilibrium of active chlorine resulted in a shift to hypochlorous acid, known to be from 80 to 100 times more disinfecting agent than hypoclorite ion.

Because of their more pronounced resistance to disinfection treatments, shown in the above-cited preliminary tests, the electrochemical inactivation of *P. aeruginosa* cultures was conducted at higher current density (100 and 150 A m^{-2}) adopting different times of treatment (from 15 to 60 min) and in further runs, enhancing the biocide activity by extending the contact time between microorganisms and oxidants in the absence of current. Final tests were then carried out by performing a sequential treatment including an anodic oxidation with and without extended contact time, followed by a cathodic treatment, as reported in Table 1.

The initial microbial culture abundance had an average density of $4.77 \times 10^8 \pm 8.15 \times 10^7$ cell mL^{-1} with an initial cell viability in the cultures of 95.3 ± 1.3 %. *P. aeruginosa* showed a behaviour strongly dependant upon the treatment conditions, as shown in Fig. 5.

In particular, the results of the treatment performed at 100 A m^{-2}, indicated a strong influence on the electrolysis time: after 30 min of electrolysis (treatment T3) 72.2 % of viable cells were finally detected, while a remarkable improvement was achieved only by increasing the time up to 60 min of electrolysis. In treatment T4, in fact, 10.7 % of residual viable cells were observed and, after recovery tests performed on specific agar (PAB), colonies development in all the recovery plates was not detected (Table 3).

An increase in the current density, though providing similar or even higher active chlorine concentrations (treatment T5 and T6) lowered the percentage of viable cells without providing a complete cell inactivation.

In treatments T3-30 and T3-60, after the electrolysis, a further contact time in the absence of current was ensured to allow the reaction to continue. As known, in fact, the

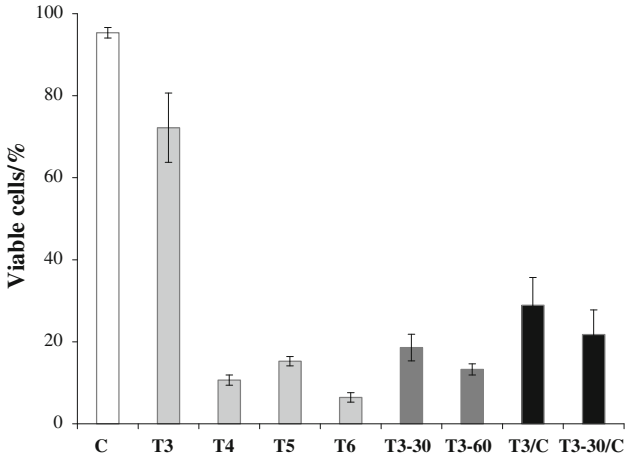

Fig. 5 Viable Cells percentage of *P. aeruginosa* after electrochemical treatment. *C* control test

Table 3 Culture development in PAB after electrochemical treatments

Treatment	Recovery plates			
	1	2	3	4
T3	y	y	y	y
T4	n	n	n	n
T5	y	y	–	–
T6	y	n	–	–
T3-30	y	y	y	y
T3-60	n	n	n	n
T3/C	y	y	y	y
T3-30/C	y (1)	y (2)	n	n

Where is no specified the colonies were uncountable, otherwise the colonies number are in brackets

y yes for colonies regrowing, *n* no regrowing, – missing data

inactivation of micro-organisms is directly related to the biocide dose, as well as the exposure time.

Results show that the percentage of viable cells decreased by increasing the exposure time with disinfection agent, and in the treatment T3-60, where 60 min of contact time after electrolysis were ensured, the complete inactivation of cells was attained. At that contact time, in fact, the amount of active chlorine in solution was about 150 and 50 mg L^{-1} in quite and stirred conditions, respectively, as shown in Fig. 6. A further increase in contact time, was less effective, probably due to the depletion of total active chlorine concentration, caused by chlorine volatilization under the acidic conditions established.

To further improve the biocide removal effectiveness, a combined treatment was also tested (T3/C and T3-30/C) when the treated anodic solution was moved to the cathodic compartment.

Adopting such a sequence, the positive effects of an additional passage of current, and the contact with other oxidising species, such as hydrogen peroxide as well as radical species, electro-generated in the cathodic oxygen reduction, were expected to enhance the overall treatment efficiency.

Results show that the cathodic post treatment allowed to obtain a substantial reduction in the percentage of viable cells (for treatment T3, from 72.2 to 28.9 %), though this option of treatment resulted to be less effective than an increase in the electrolysis time. A further improvement was achieved introducing a contact time in the absence of current between the anodic and cathodic treatment (treatment T3-30/C). Though 21.8 % of viable cells were finally detected, the combined treatment was found to be particularly effective in preventing cells from regrowing, as shown in Table 3, where it can be noticed that cells regrowing was none or negligible, considering that only one and two colonies were detected in the positive test. By comparing the data reported for all the tested treatment in the same table, similar results were only achieved in treatments T4 and T3-60, where longer treatment times and/or higher current demand were required.

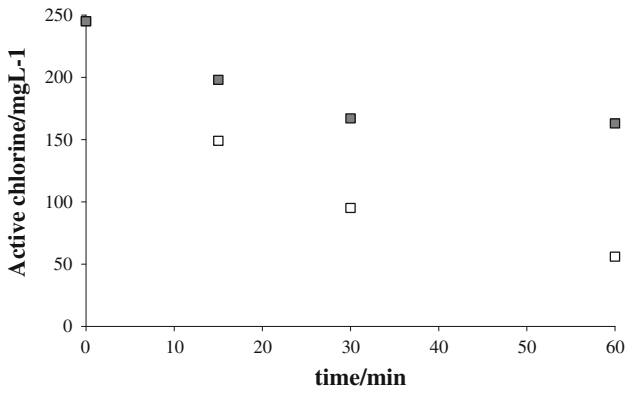

Fig. 6 Evolution of active chlorine concentration with (*empty squares*) and without (*full squares*) stirring in the absence of current

The combined treatment also allowed matching two important goals required by ballast water treatment [11], since a final circumneutral pH, and the total removal of active chlorine by reaction with the electro-generated hydrogen peroxide were attained, according to the reaction:

$$HOCl + H_2O_2 \rightarrow O_2 + H_2O + H^+ + Cl^- \quad (7)$$

as reported in a previous study [37].

However, further investigations are clearly required to assess in details all the possible inorganic by-products that can be formed during electrolysis of seawater when a BDD is used. Regarding perchlorate, preliminary tests (data not shown) confirmed that in a large excess of chloride, especially when low-current densities and relatively short treatment times were adopted, the formation of such a species resulted strongly inhibited, as confirmed in literature data [38].

4 Conclusions

The development of a combined treatment for the disinfection of ballast water involving a sequential anodic and cathodic process was investigated. The process was tested for the inactivation of two dinoflagellates responsible for algal blooms, *A. minutum* Halim and *A. taylori* Balech., and on the pathogenic marine bacterium *P. aeruginosa*.

The results of the experimental tests showed that the anodic treatment with BDD due to the combined effect of acidic pH, electric field and active chlorine, resulted in a strong inactivation effect on dinoflagellates even at low times, 15 min and low-current densities, 50 A m^{-2}. Excellent performances were also observed in the treatment of *P. aeruginosa* that resulted totally inactivated after 60 min at 100 A m^{-2}.

The adoption of a further contact time in the absence of electric charge enhanced the overall disinfection efficiency and reduced current demand.

The anodic treatment in a divided cell allowed the anode to quickly reach the acidic pH conditions that contributed to killing the species, shifting the equilibrium towards the powerful HClO species, while ensuring a low-active chlorine concentration. The separate reactor led also to cathodic accumulation of hydrogen peroxide.

The sequence adopted, involving the initial anodic oxidation followed by a contact time in the absence of current, and the final cathodic treatment, enabled total inactivation of the investigated species, final pH neutrality and removal of residual active chlorine by reaction with electro-generated hydrogen peroxide, without any external addition of

chemicals. However, further studies are required in the view of the scale up of the process, to assess materials durability and inorganic by-products electro-generation.

Acknowledgments This study was supported by the Italian Ministry of University and Research (PRIN 2008).

References

1. Nanayakkara KGN, Zheng Y-M, Alam AKMK, Zou S, Chen JP (2011) Mar Pollut Bull 63:119–123
2. McCarthy SA, Khambaty FM (1994) Appl Environ Microbiol 60:2597–2601
3. Ruiz GM, Rawlings TK, Dobbs FC, Drake LA, Mullady T, Huq A, Colwell RR (2000) Nature 408:49–50
4. Gregg MD, Hallegraeff GM (2007) Harmful Algae 6:567–584
5. Haberkorn H, Hégaret H, Marie D, Lambert C, Soudant P (2011) Harmful Algae 10:463–471
6. Hallegraeff GM (1998) Mar Ecol Prog Ser 168:297–309
7. Hallegraeff GM, Bolch CJ (1992) J Plankton Res 14:1067–1084
8. Chu KH, Tam PF, Fung CH, Chen QC (1997) Hydrobiologia 352:201–206
9. Galil BS, Hulsmann N (2002) In: Leppa-koski E, Gollasch S, Olenin S (eds) Invasive aquatic species of Europe. Distribution, impacts and management. Kluwer Academic, Dordrecht, pp 508–511
10. McCarthy HP, Crowder LB (2000) Biol Invasions 2:221–222
11. IMO (2004) International convention for the control and management of ships' ballast water and sediments. BWM/CONF/36. p 36
12. Kuzirian AM, Terry ECS, Bechtel DL, James PL (2001) Biol Bull 201:297–299
13. Cangelosi A (2002) In: Leppakoski E, Gollasch S, Olenin S (eds) Invasive aquatic species of Europe. Distribution, impacts and management. Kluwer Academic, Dordrecht, pp 511–519
14. Rigby GR, Hallegraeff GM, Taylor AH (2004) J Mar Environ Eng 7:217–230
15. Oemcke DJ, van Leeuwen J (2005) Water Res 39:5119–5125
16. Tsolaki E, Diamadopoulos E (2010) J Chem Technol Biotechnol 85:19–32
17. Da Pozzo A, Petrucci E, Merli C (2008) J Appl Electrochem 38:997–1003
18. Petrucci E, Montanaro D, Di Palma L (2012) Chem Eng Trans 28:91–96
19. Jeong J, Kim C, Yoon J (2009) Water Res 43:895–901
20. Da Pozzo A, Di Palma L, Merli C, Petrucci E (2005) J Appl Electrochem 35:413–419
21. Alonso JL, Mascellaro S, Moreno M, Ferrús MA, Hernández J (2002) Appl Environ Microbiol 68:5151–5154
22. Lahtinen SJ, Gueimonde M, Reinikainen JP, Salminen SJ (2009) Appl Microbiol Biotechnol 84:1137–1147
23. Veldhuis MJW, Kraay GW, Timmermans KR (2001) Eur J Phycol 36:167–177
24. Veldhuis MJW, Fuhr F, Boon JP, Ten Hallers-Tjabbers CC (2006) Environ Technol 27:909–921
25. Garvey M, Moriceau B, Passow U (2007) Mar Ecol Prog Ser 352:17–26
26. Llaveria G, Figueroa RI, Garcés E, Berdale E (2009) J Phycol 45:1106–1115
27. Drake LA, Steinberg MK, Riley SC, Robbins SH, Nelson BN, Lemieux E (2010) Development of a method to determine the number of living organisms $\geq$10 μm and <50 μm (nominally protists) in ships' ballast water: a combination of two vital, fluorescent stains. Naval Research Laboratory, Washington, pp 20375–25320
28. Booth BC (1987) Bot Marina 30:101–108
29. Pouneva I (1997) Bul J Plant Physiol 23:67–76
30. Kester DR, Duedall IW, Connors DN, Pytkowicz RM (1967) Limnol Oceanogr 12:176–179
31. Guillard RRL (1975) In: Smith WL, Chanley MH (eds) Culture of marine invertebrate animals. Plenum, New York, pp 26–60
32. Gilbert F, Galgani F, Cadiou Y (1992) Mar Biol 112:199–205
33. Jones KH, Senft JA (1985) J Histochem Cytochem 33:77–79
34. Reavie ED, Cangelosi AA, Allinger LE (2010) J Great Lakes Res 36:540–547
35. Yu W, Dodds WK, Banks MK, Skalsky J, Strauss EA (1995) Appl Environ Microbiol 61:3367–3372
36. Dorsey J, Yentsch CM, Mayo S, McKenna C (1989) Cytometry 10:622–628
37. Held M, Halko DJ, Hurst JK (1978) J Am Chem Soc 100:5732–5740
38. Oh BS, Oh SG, Hwang YY, Yu HW, Kang J-W, Kim IS (2010) Sci Total Environ 408:5958–5965